ON THE IMPORTANCE OF PHYSIOLOGICALLY BALANCED SOLUTIONS FOR PLANTS.[1]

I. MARINE PLANTS.

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RINGER demonstrated that animal tissues live longer in a solution of NaCl to which a small amount of KCl and $CaCl_2$ is added than in a solution of NaCl alone. Various explanations of this fact were given by different investigators, all of whom, however, agreed upon the essential point that KCl and $CaCl_2$ are essential for the maintenance of life.

HOWELL assumed that $CaCl_2$ is the stimulus for the heart beat, while NaCl is an indifferent substance, necessary only for the maintenance of osmotic pressure. Similarly RINGER concluded that Ca is the stimulus for the systole, while K is necessary for the diastole of the heart beat.

HERBST made experiments on the influence of the composition of the sea water on sea urchin eggs, eliminating in each successive experiment a different constituent of the sea water. He found that the eggs would not develop in any solution which did not contain all the salts of the sea water. From this he concluded that each of the salts found in sea water is necessary for the development of the egg. LOEB called this view in question as the result of his experiments on Fundulus. He found that this marine fish cannot live in a pure NaCl solution of the same osmotic pressure as the sea water, but that it can live indefinitely in a mixture of NaCl, KCl, and $CaCl_2$, in the same proportions in which these salts are contained in sea water. The fish can also live indefinitely in distilled water. This proves that it does not need any of the three salts mentioned for the maintenance of its life, and that the Ca and K are only required to overcome the poisonous effects which would be produced by the NaCl if it alone were present in the solution (at the above mentioned concentration).

[1] I wish here to express my sincere thanks to Professor LOEB, who kindly placed the facilities of his laboratory at my disposal and assisted me in every way during these investigations.

It is noteworthy that the Ca and K, which are added to inhibit the toxic effect of NaCl, are themselves poisonous at the concentration at which they are here employed.

These antagonistic effects of Ca and K toward a pure NaCl solution were illustrated still more strikingly in experiments on the egg of Fundulus. The newly fertilized eggs of this fish develop equally well in sea water and in distilled water, but die in a pure $m/2$ NaCl solution without forming an embryo. If, however, a small but definite amount of a salt with a bivalent kation, even of such poisonous salts as $BaCl_2$, $ZnSO_4$, and $Pb(CH_3\text{-}COO)_2$, is added, the eggs will produce embryos. From these and similar observations LOEB was led to formulate his conception of the necessity of physiologically balanced salt solutions, in which are inhibited or counteracted the toxic effects which each constituent would have if it alone were present in the solution.

The blood, the sea water, and to a large extent RINGER'S solution, are such physiologically balanced salt solutions. The observations of HERBST, as well as those of RINGER, are easily explained on this basis. The fact that the elimination of any one constituent from the sea water makes the solution unfit to sustain life does not prove that the eliminated substance is needed by the animal for any purpose other than to counteract the poisonous action of some other constituent of the solution.

Botanists have not thus far made use of these conclusions, for the obvious reason that facts similar to those mentioned above have not been observed in plants. I have recently made a number of experiments which show that there exist in plants phenomena similar to those observed by LOEB on Fundulus and other marine animals.

The species of marine plants chosen for investigation may be divided into two groups:

Group 1 comprises plants which can live a long time in distilled water. It includes the following: BLUE-GREEN ALGAE, Lyngbya aestuarii; GREEN ALGAE, Enteromorpha Hopkirkii; FLOWERING PLANTS, Ruppia maritima.

Group 2 is composed of plants which quickly die in distilled water. It includes the following: GREEN ALGAE, Enteromorpha intestinalis; BROWN ALGAE, Ectocarpus confervoides; RED ALGAE,

Ptilota filicina, Pterosiphonia bipinnata, Iridaea laminarioides, Sarcophyllis pygmæa, Nitophyllum multilobum, Porphyra naiadum, Porphyra perforata, Gelidium sp., Gymnogongrus linearis, Gigartina mammillosa.[2]

If plants of either group be placed in a solution of pure sodium chlorid (isotonic with sea water), they die in a short time. This might be attributed to the lack of certain salts which are necessary for their metabolism, rather than to the toxicity of the sodium chlorid. In the case of the plants of Group 1 there can be no doubt on this point, for these plants live a long time in distilled water. If we add pure sodium chlorid to the distilled water it kills them in a very short time. An inspection of the tables will show that these plants in their behavior toward sodium chlorid and other salts, closely agree with those of Group 2, which can live but a short time in distilled water. Sodium chlorid is certainly toxic to the first group, and there can be little doubt that it is so to the second group as well.

The plants of the first group were found in a ditch in a salt marsh through which the tide ebbs and flows; there is always a foot or so of water even at low tide. The salt content of the water fluctuates around a mean of approximately 2.3 per cent.

The plants of the second group were collected at the entrance to San Francisco Bay, where the salt content of the water fluctuates about a mean which is probably not far from 2.7 per cent. The only exceptions are *Enteromorpha intestinalis* and *Ectocarpus confervoides*, which came from wharves in the bay, where the mean salt content is about 2.3 per cent.

All the plants used in the experiments were transferred from the sea water directly to distilled water. After rinsing in this they were placed in glass dishes, each containing 200^{cc} of the solution to be tested. The dishes were then covered with glass plates to exclude dust and check evaporation. Only a small amount of material was placed in each dish. The temperature during the experiments did not vary far from 18° C.

Artificial sea water was prepared[3] according to VAN 'T HOFF'S

[2] The determinations were kindly made by Professor SETCHELL.

[3] The water used was distilled in glass only and the first part of the distillate rejected. The purity of each salt was carefully tested before using.

formula[4] as follows: 1000^{cc} NaCl, $3m/8$; 78^{cc} $MgCl_2$, $3m/8$; 38^{cc} $MgSO_4$, $3m/8$; 22^{cc} KCl, $3m/8$; 10^{cc} $CaCl_2$, $3m/8$.[5]

This closely approximates the bay water. The plants thrive almost as well in it as in sea water, especially when a very little $NaHCO_3$ or $KHCO_3$ is added to produce a neutral or faintly alkaline reaction.

A series of solutions was tried, beginning with pure NaCl $3m/8$ and adding to it in turn $MgCl_2$, KCl, and $CaCl_2$, either singly or in combination, in the proportions given above. These salts were also used in pure solutions of the same concentration at which they exist in the artificial sea water described above.

It should be said that little difficulty was experienced in determining the death point with sufficient precision. The color reactions and the microscopic appearance of the cells allowed this to be done with sufficient accuracy, so that the results were not in doubt on this account.

The results of the experiments are set forth in the tables. The figures represent the average of four parallel series carried on simultaneously. A control series was also carried on in which each solution was made faintly alkaline by the addition of $NaHCO_3$, $KHCO_3$, or $Ca(OH)_2$. This had a beneficial effect during the first two or three days of the experiment, but the final results were practically the same as in the other series.

From a consideration of the results for Group 1 we may draw the following conclusions.

1. The plants die much sooner in a pure sodium chlorid solution (isotonic with sea water) than in distilled water. The poisonous effect of the NaCl largely disappears if we add a little $CaCl_2$ (10^{cc} $CaCl_2$ $3m/8$ to 1000^{cc} NaCl $3m/8$); in this mixture the plants live nearly as long is in distilled water. Addition of KCl to this mixture enables them to live longer than in distilled water. Further addition of $MgCl_2$ and $MgSO_4$ enables them to live practically as long as in sea water.

[4] VAN'T HOFF, J. H., Physical chemistry in the service of the sciences 101. Univ. of Chicago Press, 1903.

[5] This corresponds approximately to the proportion of Ca in the sea water of the bay.

TABLE I.

DURATION OF LIFE IN DAYS.

Culture solution.	Group 1			Group 2		
	Lyngbya aestuarii	Enteromorpha Hopkirkii	Ruppia maritima	Ptilota filicina	Pterosiphonia bipinnata	Iridæa laminarioides
Sea water (total salts 2.7 %)	95	150+	150+	11	$24\frac{1}{2}$	24
Artificial sea water: 1000 cc NaCl $3m/8$; 78 " $MgCl_2$ "; 38 " $MgSO_4$ "; 22 " KCl "; 10 " $CaCl_2$ "	90	150+	150+	$10\frac{1}{2}$	$24\frac{1}{2}$	23
Distilled water.........	30	30	80	1	$3\frac{1}{2}$	$2\frac{1}{2}$
Tap water.............	32+	36	85	$2\frac{1}{2}$	$9\frac{1}{2}$	10
NaCl $3m/8$	22	15	23	$1\frac{1}{4}$	$3\frac{1}{2}$	4
1000 cc NaCl "; 10 " $CaCl_2$ "	29	23	65	$2\frac{1}{2}$	6	5
1000 " NaCl "; 22 " KCl "; 10 " $CaCl_2$ "	35	32	88	$3\frac{1}{2}$	10	9
1000 " NaCl "; 78 " $MgCl_2$ "; 10 " $CaCl_2$ "	29	23	45	3	6	6
1000 " NaCl "; 78 " $MgCl_2$ "; 22 " KCl "	25	$13\frac{1}{2}$	30	2	4	4
1000 " NaCl "; 22 " KCl "	23	$13\frac{1}{2}$	23	1	2	5
1000 " NaCl "; 78 " $MgCl_2$ "	$22\frac{5}{6}$	$13\frac{1}{2}$	25	$1\frac{1}{2}$	2	2
1000 " Dist. H_2O; 78 " $MgCl_2$ "	$15\frac{1}{2}$	$16\frac{1}{2}$	19	1	2	$2\frac{1}{4}$
1000 " Dist. H_2O; 38 " $MgSO_4$ "	$17\frac{1}{2}$	13	23	1	2	2
1000 " Dist. H_2O; 22 " KCl "	21	$13\frac{1}{2}$	56	1	$1\frac{5}{6}$	$5\frac{1}{3}$
1000 " Dist. H_2O; 10 " $CaCl_2$ "	26+	$12\frac{1}{2}$	58	$2\frac{1}{2}$	5	2

TABLE II.

DURATION OF LIFE IN DAYS. GROUP 2.

CULTURE SOLUTION.	Enteromorpha intestinalis	Ectocarpus confervoides	Sarcophyllis pygmæa	Nitophyllum multilobum	Porphyra naiadum	Porphyra perforata	Gelidium sp.	Gymnogongrus linearis	Gigartina mammillosa
Sea water (total salt 2.7 %.)	240	25	11	$4\frac{1}{2}$	6	21	33+	11	11
Artificial sea water: 1000 cc NaCl $3m/8$; 78 " $MgCl_2$ "; 38 " $MgSO_4$ "; 22 " KCl "; 10 " $CaCl_2$ "	220	20	$7\frac{1}{2}$	$4\frac{1}{2}$	6	20	33+	10	$9\frac{1}{4}$
Distilled water	3	$1\frac{1}{4}$	$1\frac{5}{6}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$3\frac{1}{3}$	$1\frac{5}{6}$	$2\frac{1}{2}$	$3\frac{1}{2}$
Tap water	10	$2\frac{1}{4}$	$3\frac{3}{4}$	$3\frac{3}{4}$	$2\frac{1}{2}$	$4\frac{1}{2}$	$5\frac{1}{2}$	$4\frac{1}{2}$	$5\frac{1}{4}$
NaCl $3m/8$	$4\frac{3}{4}$	$\frac{2}{3}$	$1\frac{2}{3}$	$\frac{5}{6}$	$2\frac{1}{6}$	3	3	$5\frac{5}{6}$	$2\frac{1}{2}$
1000 cc NaCl "; 22 " KCl "; 10 " $CaCl_2$ "	68	8	$5\frac{1}{2}$	$3\frac{1}{2}$	5	$14\frac{1}{6}$	33+	9	6
1000 " Dist. H_2O; 22 " KCl "	$4\frac{3}{4}$	4	$1\frac{5}{6}$	..	$4\frac{3}{4}$	3	3	4	3

2. The pure solution of each of the salts added to inhibit the poisonous effects of NaCl is itself poisonous at the concentration at which it exists after its addition, since the plants die in such a solution much sooner than in distilled water.[6] A mixture of solutions which are individually poisonous produces a medium in which the plants live indefinitely.

That the plants die so quickly in solutions containing a single salt might be attributed to the fact that the osmotic pressure of some of these solutions is much lower than that of sea water. This supposition is disproved by the fact that in general the plants live longer in tap water than in any solution containing but a single salt, although the tap water has a lower osmotic pressure than that of any solution used in the experiments. (The plants of Group 1 live longer in distilled water also. The tap water is to be regarded as a physi-

[6] This statement does not apply in all cases to $CaCl_2$, which is the least toxic of the salts employed and for some forms quite harmless in dilute solutions.

ologically balanced solution; this will be more fully discussed in the second portion of the paper.)

3. The poisonous effect of NaCl is inhibited little or not at all by KCl or $MgCl_2$ added singly.

4. The combination $NaCl+KCl+CaCl_2$ is superior to $NaCl+MgCl_2+CaCl_2$, but the latter is better than $NaCl+MgCl_2+KCl$.

5. These effects must be due to the metal ions, since the anion is in nearly all cases the same.

The plants of Group 2 agree with those of Group 1 except in their behavior toward distilled water.

Essentially similar results were obtained from the study of fresh water algae and other plants, the details of which will be given in the second part of this paper.

These results agree in striking fashion with those obtained from the study of marine[7] and freshwater animals[8].

The combination $NaCl+KCl+CaCl_2$ (in the same proportions as in sea water) seems to be quite generally beneficial for animals and plants.

We may in conclusion briefly consider the effects of concentrated solutions. A series of experiments were made on *Enteromorpha Hopkirkii* in which the plants were placed in dishes with a very little sea water. This quickly evaporated, so that the plants became covered with salt crystals in 24 to 48 hours. In this condition some of them remained alive for about 150 days. This means that Enteromorpha plants which remain alive only 15 days in $3m/8$ NaCl solution can live 150 days in an NaCl solution of 10 to 12 times higher concentration, provided the other salts of the sea water are present in the solution (at corresponding concentration) to inhibit the toxic effect of NaCl. Experiments on Lyngbya, Ptilota, and Pterosiphonia gave essentially the same results.

In view of these results, and others of a similar character shortly to be published, it appears certain that physiologically balanced salt solutions have the same fundamental importance for plants as for animals.

7 LOEB, Pflüger's Archiv **107**:252. 1905, and the literature there cited.

8 OSTWALD, Pflüger's Archiv **106**:568. 1905. Univ. of California Publications, Physiology **2**:163. 1905.

RESULTS.

1. Each of the salts of the sea water is poisonous where it alone is present in solution.

2. In a mixture of these salts (in the proper proportions) the toxic effects are mutually counteracted. The mixture so formed is a physiologically balanced solution.

3. Such physiologically balanced solutions have the same fundamental importance for plants as for animals.

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